Neuroendocrine Control of Metabolism of a Pulmonate Snail, *Ariophanta* Sp.

In recent years considerable attention has been focussed on the study of neurosecretion in a number of molluscs. Several workers have reported the occurrence of hormonal substances in the ganglia of central nervous system[1-6], in the optic tentacles[7-9], and gonads[10] of some molluscs. Very little is known about the neuroendocrine control of metabolism of molluscs. Factors controlling egg-production in *Arion*[11], *Aplysia californica*[12,13], and in some lamellibranchs[14-17], water balance in *Lymnaea*[18] and cardiac activity[19,20] in a few molluscs have been reported. Presence of a 'hyperglyceamic' factor in the albumin gland of a snail has been reported[21]. The present work is aimed at showing the possible role of the neurosecretions of the central nervous system of *Ariophanta* Sp. on the regulation of metabolism.

Materials and methods. Ariophanta Sp. of uniform size were collected and stocked in glass containers. The snails were used in the experiment 24 h after collection to minimize the influence of factors of alimentary origin on the major blood constituents.

Preparation of extracts. 14 nerve-rings were carefully isolated and homogenized in 14.0 ml of distilled water. 14 nerve-rings were similarly isolated and cut into dorsal halves to contain cerebral ganglia, and ventral halves to contain visceral, pleural, pedal and parietal ganglia. The two groups of dorsal and ventral halves were homogenized separately in 14.0 ml of distilled water. The homogenates were centrifuged at 1500 rpm for 10 min and the supernatents were used as nerve-ring extract, cerebral ganglia extract, and ventral ganglia extract.

Snails of uniform size were grouped into 3 experimental sets and 1 control set. The 3 experimental sets received each 1 type of extract. 0.2 ml of extract was injected into the foot, and at intervals of 30, 60, 90, and 120 min after injection blood was collected from 8 snails. Control set of snails received 0.2 ml of distilled water. Blood glucose was estimated using Glucostat enzymatic glucose reagent supplied by Worthington Biochemical Corporation, New Jersey. Free amino acids in the blood were estimated using Folin-Phenol reagent[22]. Blood sugars were estimated by the anthrone method[23].

Results. The Table gives the major blood constituents in the normal snail. Figures 1 and 2 show the effect of various extracts on these constituents. Although significant, the changes in the carbohydrate levels in the control set are not so marked as are the changes due to the injection of the extracts.

Free amino acid and carbohydrate levels in the blood of normal *Ariophanta* Sp.

S. No.	No. of estimations	Blood constituents	Blood (mg/100 ml) Mean ± S.D.
1.	12	Free amino acids	20.76 ± 4.82
2.	12	Total sugars	40.3 ± 8.15
3.	12	Glucose	5.9 ± 1.58

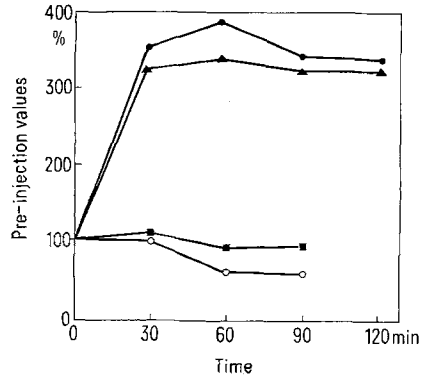

Fig. 1. Effect of central nervous tissue extracts on the blood free amino acid levels in *Ariophanta* Sp. expressed in % pre-injection values as a function of time. ●—●, cerebral ganglia; ○—○, nerve ring; ▲—▲, ventral ganglia; ■—■, control

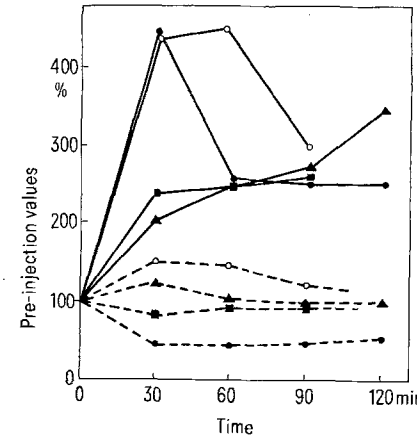

Fig. 2. Effect of central nervous tissue extracts on the blood sugar and blood glucose levels in *Ariophanta* Sp. expressed in percent pre-injection values as a function of time. —, blood glucose; ----, blood sugar; ●, cerebral ganglia; ▲, ventral ganglia; ○, nerve ring; ■, control.

1 R. NAGABHUSHANAM and T. SWARNAMAYEE, Nucleus *7*, 67 (1964).
2 R. NAGABHUSHANAM, J. mar. Ass. India 5, 316 (1963).
3 H. H. BOER, Acta. physiol. pharmac. néerl. *12*, 100 (1963).
4 I. B. BARANYI, Acta. biol. hung. *13*, suppl. 5, 63 (1963).
5 I. B. BARANYI and J. SALANSKI, Acta. biol. hung. *13*, 371 (1963).
6 R. NAGBHUSHANAM and T. SWARNAMAYEE, J. Anim. Morph. Physiol. *10*, 171 (1963).
7 N. J. LANE, Q. Jl. microsc. Sci. *103*, 211 (1962).
8 N. J. LANE, *Comparative Neurochemistry* (Pergman Press, Oxford 1964).
9 V. R. MEENAKSHI and B. T. SCHEER, Comp. Biochem. Physiol. *29*, 841 (1969).
10 P. LAVIOLETTE, C. r. Acad. Sci. Paris *231*, 1567 (1950).
11 D. PELLUET and N. J. LANE, Can. J. Zool. *39*, 789 (1961).
12 I. KUPFERMANN, Nature, Lond. *216*, 814 (1967).
13 I. KUPFERMANN, J. Neurophysiol. *33*, 877 (1970).
14 R. NAGABHUSHANAM, Indian. J. exp. Biol. *1*, 161 (1963).
15 R. NAGABHUSHANAM, Indian J. exp. Biol. *2*, 1 (1964).
16 R. NAGABHUSNAHAM, Curr. Sci. *33*, 215 (1964).
17 L. J. ANTHEUNISSE, Arch. neerl. Zool. *15*, 237 (1963).
18 J. LEVER, J. JANSEN and T. A. DE VLIEGER, nederl. Akad. Wet. Proc. *C-64*, 531 (1961).
19 C. P. JAEGAR, Comp. Biochem. Physiol. *17*, 409 (1966).
20 R. B. HILL and W. N. THIBAULT, Comp. Biochem. Physiol. *24*, 19 (1968).
21 C. K. GODDARD, P. I. NICOL and J. F. WILLIAMS, Comp. Biochem. Physiol. *11*, 351 (1964).
22 M. L. ANSON, J. gen. Physiol. *22*, 70 (1938).
23 J. H. ROE, J. biol. Chem. *212*, 335 (1955).

Injection of cerebral ganglia extract results in a sharp fall in the total blood sugars. There is a significant rise in the glucose level within 30 min and a decline later (Figure 2). This suggests the presence of a strong 'hypoglyceamic' factor and a 'hyperglyceamic' factor in the cerebral ganglia. The effect of ventral ganglia extract is a rise in the blood sugar level which returns to normal level after 1 h. There is a gradual significant rise in the blood glucose level as well, suggesting the presence of 'hyperglyceamic' factors in the ventral ganglia. When nerve-ring extract is injected, the 'hyperglyceamic' factors of cerebral and ventral ganglia predominate. Evidently there is some factor in the nerve-ring extract supressing the effect of 'hypoglyceamic' factor of the cerebral ganglia. Both cerebral and ventral ganglia extracts cause a sharp rise in the free amino acid levels suggesting the presence of factors responsible for protein catabolism. Significant lowering of free amino acid levels upon injection of nerve-ring extract shows that these factors are supressed in the extract (Figure 1).

Discussion. Not much is known about the neuroendocrine regulation of metabolism in molluscs. The presence of a 'hyperglyceamic' factor in the albumin gland of *Helix aspersa* has been suggested[21]. The present work indicates that metabolism of the snail *Ariophanta* Sp. is highly controlled and regulated. The data suggest that there is a complicated interplay of different controlling factors from the central nervous system in the regulation of metabolism. The presence of two 'hyperglyceamic' factors one for glucose, another for sugars, and a strong 'hypoglyceamic' factor in the central nervous system indicates that the carbohydrate metabolism is highly regulated in this snail. This agrees with the earlier work where it has been shown that the metabolism of the tissues of *Ariophanta* is carbohydrate-oriented[24]. The presence of principles influencing blood free amino acid levels suggests that protein metabolism is also regulated by the central nervous system[25].

Résumé. Contrôle neuroendocrinien du métabolisme d'un *Ariophanta* Sp., mollusque pulmoné. Les résultats indiquent la présence de deux facteurs hyperglycémiques dans le système nerveux central, l'un pour le glucose et l'autre pour les autres sucres. Les ganglions cérébraux contiennent un facteur hypoglycémique très actif et des facteurs qui sont responsables du catabolisme des protéines.

O. V. Subramanyam

Department of Zoology, Government College, Gudur (Andhra Pradesh, India), 19 June 1972.

[24] R. Ramamurthy and O. V. Subramanyam, in preparation (1971).

[25] The author wishes to thank Dr. R. Ramamurthy, S.V. University, Tirupati and Dr. K. V. Naidu for all the encouragement.

Induced Seed Protein Mutant of Barley

In recent years an intense search for protein-improved barleys has been carried out. A lysine increase is especially searched for because, when used as feed for non-ruminants, lysine is the first limiting essential amino acid of barley. Many variants have been found[1,2] with only modest protein improvements, followed by yield decreases of approximately 20% when compared with normal trade varieties. One of the most promising high-lysine barleys found is Hiproly[3,4]. It has 1. a genetically dependent 20 to 30% increase in lysine per 16 g of nitrogen, 2. modest changes in protein composition and in the content of the remaining amino acids[5] and 3. a yield depression of approximately 60%, based on single plants and compared with a normal lysine, high yielding variety[4].

Among the protein variants found by us, one seems really extraordinary. The aim of this paper is to report on this variant and make it available to others.

The Risø mutant 1508 was found in 1970 by screening for deviating relative dye-binding capacity (DBC)[6] in the Danish two-rowed spring barley variety Bomi treated with ethyleneimine. After a preliminary test of the mutants' protein composition in 1971, a line of mutant 1508 was established and compared with Bomi in 1972 under normal field conditions. The mutant was about 10% inferior to Bomi in grain yield. Apart from slightly smaller seeds, mutant 1508 resembles the parent variety very closely in performance.

The lysine content of the protein ($6.25 \times N$) varied from 5.18 to 5.42% in 4 replicates of mutant 1508 as compared with a variation from 3.64 to 3.82% in Bomi. The results given below (Tables I and II) derive from analysis on one replicate of the field grown material in 1972. Investigations carried out on single plants from 1971 gave similar results.

The amino acid composition of the mutant proteins, as determined by ion exchange chromatography (Table I), shows, besides the change in lysine, a 36% increase in threonine, which is supposed to be the second limiting essential amino acid. Also the contents of His, Arg, Asp, Gly and Ala have increased considerably, whereas especially the contents of Glu and Pro and, to a smaller extent, of Cys and Phe have decreased compared with the parent variety.

Barley proteins are divided into 3 major groups: 1. albumins/globulins, 2. prolamines and 3. glutelins[7]. An important aim in breeding for higher nutritional quality is to minimize the content of the lysine-poor prolamine fraction. Fractionation[8] of 2-gram samples of ground, defatted seeds (10% water) showed that the contents of albumin/globulin in the mutant increased from 27% to 46% of the total protein ($N \times 6.25$) (Table II), while the prolamine content decreased from 29% in Bomi to 9% in the mutant. The glutelin content was 39% in both. Higher amounts of non-protein nitrogen components in the mutant are indicated by the reduced percentage of nitrogen recovered as amino acids in the mutant (Table II).

The lysine content of the prolamines and of the glutelins are 192% and 36% above the respective values for the

[1] H. Doll, in *Induced Mutations and Plant Improvement* (IAEA, Vienna 1972), p. 331.

[2] J. Ingversen, A. J. Andersen, H. Doll and B. Køie, in *Nuclear Techniques for Seed Protein Improvement* (IAEA, Vienna 1973), p. 193.

[3] A. Hagberg and K. E. Karlsson, in *New Approaches to Breeding for Improved Plant Protein* (IAEA, Vienna 1969), p. 17.

[4] L. Munck, K. E. Karlsson, A. Hagberg and B. O. Eggum, Science *168*, 985 (1970).

[5] L. Munck, K. E. Karlsson and A. Hagberg, in *Barley Genetics*, II. Proc. Sec. Int. Barley Gen. Symp. (Washington State University Press 1971), p. 544.

[6] R. Mossberg, in *New Approaches to Breeding for Improved Plant Protein* (IAEA, Vienna 1969), p. 151.

[7] T. B. Osborne, J. Am. chem. Soc. *17*, 587 (1895).

[8] J. Ingversen and B. Køie, Phytochemistry *12*, 73 (1973).